Attenuation of renal excretory responses to ANG II during inhibition of superoxide dismutase in anesthetized rats

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Khan MA, Islam MT, Castillo A, Majid DS. Attenuation of renal excretory responses to ANG II during inhibition of superoxide dismutase in anesthetized rats. Am J Physiol Renal Physiol 298: F401–F407, 2010. First published November 18, 2009; doi:10.1152/ajprenal.00511.2009.—To examine the functional interaction between superoxide dismutase (SOD) and NADPH oxidase activity, we assessed renal responses to acute intra-arterial infusion of ANG II (0.5 ng·kg⁻¹·min⁻¹) before and during administration of a SOD inhibitor, diethyldithiocarbamate (DETC, 0.5 mg·kg⁻¹·min⁻¹), in enalaprilat-pretreated (33 µg·kg⁻¹·min⁻¹) rats (n = 11). Total (RBF) and regional (cortical, CBF; medullary, MBF) renal blood flows were determined by Transonic and laser-Doppler flowmetry, respectively. Renal cortical and medullary tissue NADPH oxidase activity in vitro was determined using the lucigenin-chemiluminescence method. DETC treatment alone resulted in decreases in RBF, CBF, MBF; glomerular filtration rate (GFR), urine flow (V), and sodium excretion (UNaV) as reported previously. Before DETC, ANG II infusion decreased RBF (−18 ± 3%), CBF (−16 ± 3%), MBF (−5 ± 6%); P = not significant (NS), GFR (−31 ± 4%), V (−34 ± 2%), and UNaV (−53 ± 3%). During DETC infusion, ANG II also caused similar reductions in RBF (−20 ± 4%), CBF (−19 ± 3%), MBF (−2 ± 2%; P = NS), and in GFR (−22 ± 7%), whereas renal excretory responses (V: −12 ± 2%; UNaV: −24 ± 4%) were significantly attenuated compared with those before DETC. In vitro experiments, ANG II (100 µM) enhanced NADPH oxidase activity both in cortical [13,194 ± 1,651 vs. 20,914 ± 2,769 relative light units (RLU)/mg protein] and in medullary [21,296 ± 2,244 vs. 30,597 ± 4,250 RLU/mg protein] tissue. Application of DETC (1 mM) reduced the basal levels and prevented ANG II-induced increases in NADPH oxidase activity in both tissues. These results demonstrate that renal excretory responses to acute ANG II administration are attenuated during SOD inhibition, which seems related to a downregulation of NADPH oxidase in the deficient condition of SOD activity.

superoxide dismutase; NADPH oxidase; diethyldithiocarbamate; renal hemodynamics; sodium excretion

ANG II is a powerful vasoconstrictor agent that contributes to the regulation of renal function and blood pressure (22, 23, 26, 30). Elevated intrarenal ANG II level causes alterations in the renal function, leading to sodium retention and thus is involved in the development and maintenance of hypertension (22, 23). ANG II is known to induce superoxide (O₂⁻) generation via activation of NADPH oxidase activity (5). An increase in oxidative stress has been strongly suggested to be involved in the development and maintenance of ANG II-dependent hypertension (26, 30).

O₂⁻ is a product of cellular oxidative metabolism, and NADPH oxidase is one of the major sources of O₂⁻ in living tissue (1). Reactive O₂⁻ needs to be instantly reduced by the enzyme superoxide dismutase (SOD) (25). It had been demonstrated that the pretreatment with a SOD mimetic, tempol, attenuates renal hemodynamics and excretory responses to both acute (16) and chronic (9) administration of ANG II, indicating that the renal vascular and tubular actions of ANG II are partially mediated by concomitant generation of O₂⁻. Furthermore, the renal excretory responses to acute administration of an inhibitor of nitric oxide (NO) synthase were also shown to be partially attenuated by tempol treatment, indicating that NO blockade enhances O₂⁻ activity in the kidney (17). On the other hand, SOD inhibition with sodium diethyldithiocarbamate (DETC) in anesthetized dogs caused decreases in both renal cortical and medullary blood flow as well as urinary sodium excretion, which were significantly enhanced during NO synthase inhibition (15). Similar reductions in regional blood flow and sodium excretion were also noted when DETC was administered directly in the renal medulla (33). However, the role of SOD inhibition in the renal responses to ANG II administration has not yet been investigated.

We hypothesized that the activity of a SOD enzyme limits ANG II-induced increases in NADPH oxidase activity and thus prevents exacerbation of the renal vascular and tubular actions of ANG II in the condition of enhancement of the renin-angiotensin system. In the present study, we examined this hypothesis by conducting experiments in rats with consideration of two specific aims: 1) to investigate in vivo the renal responses to ANG II administration during inhibition of SOD in anesthetized rats and 2) to assess in vitro the NADPH oxidase activity in response to ANG II treatment during inhibition of SOD in rat renal tissue. Anesthetized rats pretreated with an angiotensin-converting enzyme inhibitor, enalaprilat, were used in these experiments to minimize the possible influence of alterations in endogenous ANG II during the experimental period (24). In these enalaprilat-pretreated rats, renal hemodynamic and excretory responses to acute intra-arterial administration of ANG II directly in the kidney were assessed before and during SOD inhibition with DETC administration. NADPH oxidase activity was assessed in vitro in renal tissues collected from these anesthetized rats pretreated with enalaprilat.

METHODS

All of the experimental procedures described in this study were approved by and performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee. These experiments were conducted in male Sprague-Dawley rats weighing 250–300 g body wt (8 wk old) that were purchased from Charles River Laboratories (Wilmington, MA), housed at least 3 days for aclimatization in a temperature- and light-controlled room, and allowed free access to a standard diet (Ralston-Purina, St. Louis, MO) and tap water. On the day of experiments, these rats were anesthetized with thiobutabarbital sodium (Inactin; Sigma-Aldrich) at a dose of 100 mg/kg ip. Supplemental
Table 1. Renal hemodynamic and excretory responses to ANG II before and during administration of DETC in enalaprilat-pretreated rats (n = 11)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>EN (Pre-ANG II)</th>
<th>EN + ANG II</th>
<th>EN (Post-ANG II)</th>
<th>EN + DETC</th>
<th>EN + DETC + ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>107 ± 1.7</td>
<td>102 ± 1</td>
<td>100 ± 2</td>
<td>100 ± 2</td>
<td>101 ± 2</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Renal blood flow, ml/min⁻¹·g⁻¹</td>
<td>5.4 ± 0.2</td>
<td>6.0 ± 0.3</td>
<td>4.7 ± 0.3†</td>
<td>5.2 ± 0.3</td>
<td>4.6 ± 0.3‡</td>
<td>3.7 ± 0.2§</td>
</tr>
<tr>
<td>Cortical blood flow, PU/min</td>
<td>328 ± 20</td>
<td>346 ± 21</td>
<td>288 ± 18†</td>
<td>315 ± 18</td>
<td>287 ± 16‡</td>
<td>233 ± 198</td>
</tr>
<tr>
<td>Medullary blood flow, PU/min</td>
<td>172 ± 22</td>
<td>172 ± 24</td>
<td>152 ± 15</td>
<td>148 ± 15</td>
<td>131 ± 11</td>
<td>127 ± 10</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min⁻¹·g⁻¹</td>
<td>0.7 ± 0.03</td>
<td>0.9 ± 0.06*</td>
<td>0.6 ± 0.06†</td>
<td>0.7 ± 0.05</td>
<td>0.5 ± 0.04‡</td>
<td>0.4 ± 0.02$</td>
</tr>
<tr>
<td>Urine flow, μl/min⁻¹·g⁻¹</td>
<td>9.7 ± 0.7</td>
<td>10.7 ± 0.6*</td>
<td>7.1 ± 0.5†</td>
<td>8.6 ± 0.6</td>
<td>8.0 ± 0.6</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>Urinary excretion of sodium, μmol/min⁻¹·g⁻¹</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1*</td>
<td>0.7 ± 0.06†</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.05‡</td>
<td>0.5 ± 0.06</td>
</tr>
<tr>
<td>Fractional excretion of sodium, %</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.75 ± 0.06†</td>
<td>0.9 ± 0.04</td>
<td>0.9 ± 0.04</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td>Urinary excretion of 8-isoprostane, pg/min⁻¹·g⁻¹ (n = 7)</td>
<td>3.5 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>6.0 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.3</td>
<td>3.0 ± 0.3§</td>
</tr>
<tr>
<td>Urinary excretion of nitrate/nitrite, nmol/min⁻¹·g⁻¹ (n = 7)</td>
<td>1.6 ± 0.07</td>
<td>1.7 ± 0.1</td>
<td>0.7 ± 0.08†</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. EN, enalaprilat; DETC, diethyldithiocarbamate; PU, perfusion unit. *P < 0.05 vs. control (†), vs. EN (Pre-ANG II) (‡), vs. EN (Post-ANG II) (§), and vs. EN + DETC ($).
and weighed so that the calculated parameters could be expressed per gram of kidney weight.

**In vitro measurement of renal NADPH oxidase activity.** In a separate set of experiments, kidney samples were collected from anesthetized rats pretreated with enalaprilat used in time control experiments (n = 7). After collection, these samples were snap-frozen and then kept at −80°C for in vitro measurement of NADPH oxidase activity. Determination of renal cortical and medullary NADPH oxidase activity was carried out in vitro by the lucigenin-chemiluminescence assay as described earlier (21). The NADPH oxidase activity is expressed as relative light unit/mg protein (RLU/mg protein). Protein content of the renal tissues was determined by the Bradford method using a commercially available assay kit (Bio-Rad). DETC-induced changes in luminescence in vitro were also tested in a protein-free solution to determine its nonspecific effects unrelated to O2 generation.

**Analytical procedures, calculations, and statistical analysis.** The collected blood and urine samples were analyzed for inulin and sodium/potassium concentrations. Inulin was determined by spectrophotometry, and sodium/potassium concentrations were determined by flame photometry. The value of inulin clearance was considered as glomerular filtration rate (GFR). The formula for measurement of GFR was as follows: GFR (inulin clearance) = (urinary concentration of inulin × urine volume)/plasma inulin concentration. Renal vascular resistance (RVR) was calculated by dividing the value of mean arterial pressure (MAP) with the value of RBF. Urinary concentrations of 8-isoprostanе and nitrate/nitrite were determined by enzyme immunoassay and colorimetric methods using commercially available assay kits (Cayman Chemical). All values were normalized per gram of kidney weight. Data are expressed as means ± SE. Statistical comparisons of differences in the responses were carried out by one-way ANOVA followed by appropriate (Holm-Sidak) post hoc tests. Comparison of the percent responses to ANG II infusion before and during DETC infusion was made using unpaired Student’s t-test. Differences in the mean values were deemed significant at P <0.05.

**RESULTS**

**Effects of enalaprilat administration alone on renal hemodynamics and excretory function.** These effects are summarized in Table 1. Infusion of enalaprilat in the renal artery in these rats (n = 11) did not significantly influence MAP. However, there were mild to moderate increases in renal hemodynamic and excretory parameters during enalaprilat administration in these rats. Although the changes in RBF, CBF, MBF, or urine flow (V) did not reach statistical significance, the increases in GFR and sodium excretion (UNaV) were significant. The urinary excretion of 8-isoprostanе and nitrate/nitrite (UNOXV) were not significantly influenced by enalaprilat infusion.

**Fig. 2.** Percentage (%) changes in renal blood flow (RBF; A) and renal vascular resistance (RVR; B) responses to acute ANG II in the presence and absence of superoxide dismutase (SOD) inhibition by DETC (n = 11 experiments). *P < 0.05 vs. basal.

**Fig. 3.** Percentage (%) changes in cortical blood flow (CBF; A) and medullary blood flow (MBF; B) responses to acute ANG II in the presence and absence of SOD inhibition by DETC (n = 11). *P < 0.05 vs. basal.
administration of DETC were seen markedly attenuated during antidiuretic and antinatriuretic responses to ANG II before 7% (Fig. 4A) was also not significant. However, the marked /H11002 before and during DETC treatment (31 3% vs. 27 /H11006 also not statistically different (CBF, MBF, /H11002 5% vs. 2% (Fig. 4B) and vs. before DETC (#).

Effect of DETC on renal hemodynamics and excretory function. Table 1 also summarized these results. Intra-arterial infusion of DETC directly in the kidney for 50 min decreases renal hemodynamic and excretory parameters, mostly similar to those reported in other studies (15, 32). However, there were no apparent changes in UNOXVo rU IsoV during DETC treatment (Fig. 5). It is observed that the reduction of RBF by ANG II compared with the first administration on renal parameters, mostly similar effects of DETC inhibition by DETC (V, −34 ± 3% vs. −12 ± 2% (Fig. 4B) and UNaV, −53 ± 3% vs. −24 ± 4% (Fig. 5A)). It was noted that the decrease in fractional excretion of sodium (FENa) in response to ANG II before DETC infusion (−36 ± 4%) was markedly attenuated during DETC infusion (−6 ± 11%) (Fig. 5B). However, there were no such reductions, rather slightly increased, in the responses to the second administration of ANG II compared with the first administration on renal parameters in the corresponding time-controlled experiments (n = 5). The changes in renal excretory parameters in response to the first and the second administrations of ANG II were as follows: V, 9.4 ± 0.3 to 5.4 ± 0.6 μl·min⁻¹·g⁻¹ (−42.5 ± 4.8%) and 7.4 ± 0.7 to 3.8 ± 0.2 μl·min⁻¹·g⁻¹ (−47.5 ± 3.8%); UNaV, 1.3 ± 0.1 to 0.6 ± 0.04 μmol·min⁻¹·g⁻¹ (−53.5 ± 3.4%) and 0.9 ± 0.1 to 0.4 ± 0.04 μmol·min⁻¹·g⁻¹ (−58.2 ± 3.3%); and FENa, 0.8 ± 0.03 to 0.4 ± 0.02% (−47.6 ± 1.8%) and 0.6 ± 0.03 to 0.3 ± 0.03% (−53.5 ± 3.5%). There were also similar changes in the renal hemodynamic parameters during the first and the second administrations of ANG II in these time-controlled experiments. The changes in renal hemodynamic parameters in response to the first and the second administrations of ANG II were as follows: RBF, 6.9 ± 0.6 to 4.7 ± 0.3 ml·min⁻¹·g⁻¹ (−31.5 ± 3.8%) and 5.8 ± 0.7 to 3.2 ± 0.3 ml·min⁻¹·g⁻¹ (−44.1 ± 3.5%); CBF, 391 ± 14 to 324 ± 21 perfusion units (PU); (−17.5 ± 2.9%) and 334 ± 19 to 221 ± 16 PU (−34 ± 2.3%); MBF 122 ± 9.3 to 118 ± 8 PU (−2.7 ± 2.0%) and 121 ± 0.03 to 0.4 ± 0.04% (−31.5 ± 3.8%) and 5.8 ± 0.7 to 3.2 ± 0.3 ml·min⁻¹·g⁻¹ (−44.1 ± 3.5%); CBF, 391 ± 14 to 324 ± 21 perfusion units (PU); (−17.5 ± 2.9%) and 334 ± 19 to 221 ± 16 PU (−34 ± 2.3%); MBF 122 ± 9.3 to 118 ± 8 PU (−2.7 ± 2.0%) and 121 ±

Renal responses to infusion of ANG II before and during DETC treatment. The summarized absolute results from these experiments are shown in Table 1. Comparison of the percent-age responses to ANG II on renal hemodynamics and excretory functions before and during DETC infusion were shown in Figs. 1–5. It is observed that the reduction of RBF by ANG II was similar before and during DETC treatment (−18 ± 3% vs. −20 ± 4%) (Fig. 2A). The increases in RVR caused by ANG II administration before and during DETC treatment (22 ± 6 vs. 27 ± 8%) were also not significantly different (Fig. 2B). The magnitudes of the percent reduction of CBF and MBF responses to ANG II before and during DETC treatment were also not statistically different (CBF, −16 ± 3% vs. −19 ± 3%; MBF, −5 ± 6% vs. −2 ± 2%) (Fig. 3, A and B). The difference between the magnitudes of percent GFR responses to ANG II before and during DETC treatment (−31 ± 4% vs. −22 ± 7%) (Fig. 4A) was also not significant. However, the marked antidiuretic and antinatriuretic responses to ANG II before administration of DETC were seen markedly attenuated during DETC treatment [V, −34 ± 3% vs. −12 ± 2% (Fig. 4B) and UNaV, −53 ± 3% vs. −24 ± 4% (Fig. 5A)]. It was noted that the decrease in fractional excretion of sodium (FENa) in response to ANG II before DETC infusion (−36 ± 4%) was markedly attenuated during DETC infusion (−6 ± 11%) (Fig. 5B). However, there were no such reductions, rather slightly increased, in the responses to the second administration of ANG II compared with the first administration on renal parameters in the corresponding time-controlled experiments (n = 5). The changes in renal excretory parameters in response to the first and the second administrations of ANG II were as follows: V, 9.4 ± 0.3 to 5.4 ± 0.6 μl·min⁻¹·g⁻¹ (−42.5 ± 4.8%) and 7.4 ± 0.7 to 3.8 ± 0.2 μl·min⁻¹·g⁻¹ (−47.5 ± 3.8%); UNaV, 1.3 ± 0.1 to 0.6 ± 0.04 μmol·min⁻¹·g⁻¹ (−53.5 ± 3.4%) and 0.9 ± 0.1 to 0.4 ± 0.04 μmol·min⁻¹·g⁻¹ (−58.2 ± 3.3%); and FENa, 0.8 ± 0.03 to 0.4 ± 0.02% (−47.6 ± 1.8%) and 0.6 ± 0.03 to 0.3 ± 0.03% (−53.5 ± 3.5%). There were also similar changes in the renal hemodynamic parameters during the first and the second administrations of ANG II in these time-controlled experiments. The changes in renal hemodynamic parameters in response to the first and the second administrations of ANG II were as follows: RBF, 6.9 ± 0.6 to 4.7 ± 0.3 ml·min⁻¹·g⁻¹ (−31.5 ± 3.8%) and 5.8 ± 0.7 to 3.2 ± 0.3 ml·min⁻¹·g⁻¹ (−44.1 ± 3.5%); CBF, 391 ± 14 to 324 ± 21 perfusion units (PU); (−17.5 ± 2.9%) and 334 ± 19 to 221 ± 16 PU (−34 ± 2.3%); MBF 122 ± 9.3 to 118 ± 8 PU (−2.7 ± 2.0%) and 121 ± 0.03 to 0.4 ± 0.04% (−31.5 ± 3.8%) and 5.8 ± 0.7 to 3.2 ± 0.3 ml·min⁻¹·g⁻¹ (−44.1 ± 3.5%); CBF, 391 ± 14 to 324 ± 21 perfusion units (PU); (−17.5 ± 2.9%) and 334 ± 19 to 221 ± 16 PU (−34 ± 2.3%); MBF 122 ± 9.3 to 118 ± 8 PU (−2.7 ± 2.0%) and 121 ±

Fig. 4. Percentage (%) changes in glomerular filtration rate (GFR; A) and urine flow (V; B) responses to acute ANG II in the presence and absence of SOD inhibition by DETC (n = 11). P < 0.05 vs. basal (*) and before DETC (#).

Fig. 5. Percentage (%) changes in urinary sodium excretion (UNaV; A) and fractional excretion of sodium (FENa; B) responses to acute ANG II in the presence and absence of SOD inhibition by DETC (n = 11). P < 0.05 vs. basal (*) and vs. before DETC (#).
9 to 102 ± 17 PU (−15 ± 13%); and GFR 1.1 ± 0.1 to 0.9 ± 0.01 ml·min\(^{-1}\)·g\(^{-1}\) (−23.6 ± 2.2%) and 1.0 ± 0.1 to 0.6 ± 0.03 ml·min\(^{-1}\)·g\(^{-1}\) (−38.6 ± 2.7%). These findings demonstrate that the observed reductions in the renal responses to ANG II in the presence of DETC were unlikely to be contributed by the time-dependent changes in the present study.

U\(_{ISO}\) and U\(_{NOx}\), obtained from seven rats tested in these experiments. ANG II administration markedly increased U\(_{ISO}\) before the infusion of DETC. However, during DETC infusion, U\(_{ISO}\) was decreased by ANG II administration. ANG II administration caused a decrease in U\(_{NOx}\) before DETC infusion but caused no change during DETC infusion.

Renal cortical and medullary NADPH oxidase activity in response to ANG II before and during DETC treatment. Figure 6 shows the summarized results obtained from in vitro studies. ANG II enhanced the NADPH oxidase activity in both cortical and medullary tissues (cortex, 13,194 ± 1,651 vs. 20,914 ± 2,769 RLU/mg protein and medulla, 21,296 ± 2,244 vs. 30,597 ± 4,250 RLU/mg protein) (Fig. 6, A and B). DETC treatment decreased NADPH oxidase activity both in the cortex (5,805 ± 1,688 RLU/mg protein) and in the medulla (7,921 ± 960 RLU/mg protein). These reductions in NADPH activity could not be considered being a nonspecific effect of DETC to reduced luminescence by a mechanism unrelated to \(O_2\) generation in vitro, since DETC did not reduce the luminescence value in a protein-free solution but rather increased this value (control, 84 ± 7 RLU vs. DETC, 422 ± 16 RLU; \(n = 4\)). However, when the tissues were treated with ANG II in the presence of DETC, the ANG II-mediated enhancement in NADPH oxidase activity was attenuated in both the cortex (6,494 ± 1,438 RLU/mg protein) and medulla (10,315 ± 2,261 RLU/mg protein) (Fig. 6, A and B).

DISCUSSION

The results from the present investigation demonstrate that SOD inhibition in the kidney does not alter the responses to acute administration of ANG II on RBF or GFR but shows significant attenuation of its antinatriuretic effect in rats pretreated with enalaprilat. Because SOD effectively reduces the reactive molecule of \(O_2\), its inhibition is expected to increase the tissue level of this oxygen radical, particularly during administration of ANG II, which stimulates NADPH oxidase activity to generate this radical compound in biological tissue (1). As a well-known effective inhibitor of SOD (8), the metal ion-chelating agent DETC was used in many previous studies (15, 18, 19, 33) to assess the role of cellular \(O_2\) formation and thus the role of enhanced oxidative stress in regulating different organ function, including the kidney. It was reported that administration of DETC caused decreases in RBF, GFR, and \(U_{Na}\) when infused intra-arterially in dogs (15) or intrarenally in rats (33), and these responses to DETC were attributed to enhancement of \(O_2\) activity in the kidney. In the present study, DETC administration alone in the kidney also induced similar renal effects as reported previously (15), indicating that the dose of DETC used here was effective as an inhibitor of SOD. However, contrary to our hypothesis, SOD inhibition by DETC in these present experiments did not exacerbate the renal actions of ANG II but rather attenuated its antinatriuretic action. In addition, it was also observed that DETC treatment attenuated the ANG II-induced increases in both U\(_{ISO}\) (marker for endogenous \(O_2\) activity) in vivo and the renal tissue NADPH oxidase activity in vitro. To our knowledge, this is the first in vivo study that evaluated the renal responses to acute administration of ANG II in the condition of inhibited SOD activity.
Previous studies using O₂ scavenger or inhibitor (6, 10, 16) indicated an involvement of concomitant generation of O₂ in mediating renal vasoconstrictor and excretory responses to acute administration of ANG II (6, 16). However, the contribution of possible enhancement in O₂ activity is not reflected in the renal responses to acute ANG II during SOD inhibition in the present study. This is mainly due to the fact that SOD inhibition by DETC attenuates the tissue NADPH oxidase activity and thus limits O₂ production in response to ANG II. The findings in our earlier studies (15, 16, 17) demonstrated that the changes in O₂ activity in response to acute ANG II administration or NO inhibition in the kidney exert comparatively greater influence on renal tubular than on the vascular function. Thus it is expected that the renal action of ANG II is more attenuated in excretory function than in hemodynamic function due to reductions in NADPH oxidase activity in response to SOD inhibition in the present study. These findings demonstrate that a functional deficiency in SOD activity resulted in attenuation of both basal and ANG II-stimulated increases in tissue NADPH oxidase activity in the kidney, which also correlates with the functional data showing attenuation of renal responses to ANG II. Thus these interesting results strongly suggest an interactive role between SOD and NADPH oxidase enzymes in limiting each other’s activity in the state of an enhanced renin-angiotensin system and oxidative stress.

Although an existence of such cross link between the activities of these two enzymes (NADPH oxidase and SOD) is not clearly known in the current literature, an earlier study in vitro (8) also reported that application of DETC in rat aortic rings inhibited the stimulated production of O₂ by blocking the catalytic activity of xanthine oxidase while not affecting the basal production of vascular O₂. The findings in the present study in vivo also demonstrate that DETC treatment inhibited the stimulated production of O₂ by ANG II, since it is observed that UisoV decreased during ANG II infusion in the presence of DETC, whereas it was increased before DETC administration. SOD inhibition by DETC treatment had been shown to reduce intracellular reactive oxygen species production in ANG II-treated cardiac fibroblasts (12). Supporting these observations, it was also reported that chronic administration of ANG II for 2 wk in extracellular SOD (ecSOD) knockout mice resulted in a downregulation of NADPH oxidase activity both in aortic tissue (4) and in the kidney (31). Increases in renal tissue O₂ production and UisoV because of chronic ANG II administration were also shown to be absent in ecSOD knockout mice (31). Thus these cumulative data strongly suggest that, in a deficient condition of SOD enzyme, a downregulation of NADPH oxidase activity occurs that would prevent the stimulated production of O₂ and would limit the possible exacerbation of renal functional responses to an enhanced renin-angiotensin system.

The exact mechanism of how SOD inhibition causes attenuation of NADPH oxidase activity is not clear yet. However, it had been reported that decreases in cellular SOD activity by administration of DETC in vitro decrease nuclear transcription factor-κB (NF-κB) (27, 32). In fact, the dithiocarbamate compounds such as pyrrolidine dithiocarbamates and DETC were used in previous studies (2, 13, 14) as an inhibitor of NF-κB activation to evaluate its role in mediating inflammatory responses to various tissue injury. Because ANG II-induced activation of NADPH oxidase has been linked to upregulation of NF-κB activity in many studies (7, 28, 29), it is conceivable that an inhibition of such nuclear transcription factor by DETC may suppress the activity of NADPH oxidase observed in the present study. It is also possible that, while administration of DETC effectively inhibits ecSOD, it could also be less effective in inhibiting the cytosolic or intracellular isoform of SOD (Cu-Zn SOD). It was reported that chronic ANG II administration in gene knockout mice lacking the ecSOD gene upregulated the intracellular isoform of SOD, whereas this isoform was downregulated in the corresponding wild-type strain of mice (4, 31). In an earlier study, it is also observed that, in mice lacking ecSOD, the upregulation of cytosolic isoforms of SOD offset ANG-II mediated enhancement of NAPDH oxidase activity (30). It is to be noted here that rats are usually known as a species that lacks vascular ecSOD activity (3). Thus, in the present study in rats, ANG II administration may have increased the cytosolic isoform of SOD, which offset the enhanced activity of NADPH oxidase to limit the production of tissue O₂ during DETC administration. However, further experiments with a specific protocol to examine the activity of different isoforms of SOD in response to acute ANG II administration need to be conducted to clarify this issue.

In conclusion, these results demonstrate that renal excretory responses to acute ANG II administration are attenuated during SOD inhibition. These data suggest that such attenuation of renal functional responses to ANG II is related to a downregulation of NADPH oxidase activity in the deficient condition of SOD enzyme.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


